

## ***Research Needs and Opportunities for Micro-CT of Microcirculatory Structure and Function***

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Figure 1 [1] There are two levels of CT imaging small animals – mini- and micro-CT. Mini-CT has a voxel resolution that is equivalent to whole body CT of the adult human. Thus, a 30 cm diameter human chest is represented with up to 500 pixels clinically, so a 2 cm mouse chest would be represented by 500, 40,  $\mu\text{m}$  pixels. Higher resolution scans at the microscopic level are truly micro-CT scans. I will be talking primarily about mini-CT image analysis of the microvasculature, although occasionally I will refer to the need for micro-CT imaging.

Figure 2 [2] shows the opacified bile ducts and coronary arteries of a rat and a mouse respectively. The point is that even though I will be talking primarily about the microvasculature, the bile ducts in the liver, the collecting ducts in the kidney, the salivary ducts in the salivary glands etc also can be analyzed in the same way as I will discuss for the microvasculature. X-ray- CT images generally have insufficient contrast to delineate the blood within vessels from the vessel wall, hence we need to inject an agent into the lumens that increases the lumen opacity. In the lung, which has much air in it quite a bit can be learned about the vasculature with minimal contrast whereas within bone the contrast has to be quite heavy if we are to confidently distinguish blood from bone.

Figure 3 [3], the microvasculature has basically three levels of size that relate to the vessel functional characteristics. Thus, arteries larger than 150  $\mu\text{m}$  in lumen diameter are primarily conduit vessels, the vessels 150 to 30  $\mu\text{m}$  are resistance vessels, i.e. they constrict or dilate to increase or decrease resistance to blood flow and those less than 30  $\mu\text{m}$  are vessels involved in transport of nutrients etc across the vessel wall into the surrounding tissues. These panels show the vessels greater than 30  $\mu\text{m}$ , although at  $<4$   $\mu\text{m}$  voxel resolution, we can see vessels less than 30  $\mu\text{m}$ , indeed we can see the capillaries, which are 5  $\mu\text{m}$  in diameter.

Figure 4 [courtesy of Dr. R. Chinery] illustrates how arteries respond to disease states. The left image is of a normal colonic arterial tree and on the right is the same vascular tree in the presence of intraluminal adenomas. These adenomas develop new vessels at the  $<30$   $\mu\text{m}$  level (i.e. angiogenesis) and this increases the flow through the larger arteries which in turn respond by dilating as shown here. Thus a normal vessel may reflect disease down stream.

Figure 5 [4], although we talk about arteries primarily, veins also are important –in fact the whole erectile dysfunction pharmaceutical industry depends on them! This shows the arteries and veins in a rabbit penis, left normal and right diabetic. Impotence is increased in incidence in diabetes mellitus and this finding is consistent with a microvascular mechanism as illustrated in these micro-CT images.

Figure 6 [5], Some diseases, such as high blood cholesterol levels are associated with increased microvasculature in some tissues. However, it is not clear whether this is a cause or an effect. If it is an effect, then why is high cholesterol associated with ischemia?

We can quantitate new vessel growth by identifying the preponderance of vessels of selected diameters. This can be done by “eroding” the segmented vessels until all vessels than some selected diameter have disappeared, when “redilating” that image back to its ‘original’ state. The difference between the original and processed image is an image of all small diameter vessels, presumably reflecting the level of angiogenesis if it is present.

The volume of tissue perfused by an artery is an important index as I will discuss later. This volume can be estimated by outlining a perfusion territory as defined by the visible limits of a tree, but this is generally not a workable approach.

Figure 7, an effective method involves “dilating” the vessels by adding voxels to the surface of the arterial tree. When the spaces between the branches are filled and the rate of increase of the total volume is reduced so we stop ‘dilating’ at that point. The total number of vessels now describes the volume of the tissue perfused by this vessel.

Figure 8 [6] - This shows that in a rat heart the perfused volume of heart muscle is linearly related to the cross sectional area of the perfusing artery and

Figure 9 [6] shows that this relationship, as indicated by the slope of that regression in Figure 8, increases with animal size. Interestingly this increase almost exactly matches the increase in metabolic rate with body size. A somewhat related issue is that some individuals have higher running capacity than do others. Thus a group of rats has been bred to have high running capacity [7]. This could be due to increased blood supply to a volume of muscle or due to increased efficiency of the muscle. An analysis of micro-CT images of a rat heart and leg muscle show that there is no difference in the vasculature, so it must be cellular.

Figure 10 [courtesy of Dr. M. Gössl] - A particularly interesting microvasculature is the vasa vasorum of large arteries. They perfuse the walls of arteries with lumens greater than 1 mm in diameter.

Figure 11 [courtesy of Dr. M. Zamir] shows that we can measure them. We have found that they have trees that follow branching rules just like other arteries. These trees can be used to compute resistant to blood flow and hence the pressure within such vasa as a function of their location within the arterial wall. An arterial wall has compressive forces within it so that it prevents the lumen from distending. This pressure falls off exponentially as you progress from the lumen to the outside of the wall. Hence as the vasa enter the outside of the wall and progress towards the lumen they become increasingly compressed and the pressure within their lumens become increasingly diminished. Consequently, at some point these pressures become equal and the vasa must collapse.

Figure 12 [8] - The arterial wall also can change in response to disease processes. This shows arterial wall calcification in an old rat. It is often a direct result of longstanding atherosclerosis. Additional information about arterial walls can be obtained by staining the wall in ways that

shows up various aspects of the wall structure. Osmium tetroxide stains fatty material such as cell walls and hence shows up the cellularity of this wall.

Figure 13 [9] - Some pathophysiological processes are transient and cannot be captured in real time with micro-CT imaging. Hence we solve this problem by snap freezing the tissue at selected time points during the dynamic process. Thus the movement of a radio-opaque substance across the endothelium of a blood vessel into the surrounding wall. This is made possible by this dewar chamber which consists of a double walled container with a vacuum separating the two walls. These copper walls have beryllium windows in them so that the x-ray can pass through with minimal attenuation. Specimens can be scanned in this vessel over a period of many hours without thawing.

Figure 14 - That this works well is illustrated by this 3D image of some genuine Minnesota snow and several of the snowflakes extracted from the 3D image in left panel.

In anesthetized animals we inject contrast agent into a vessel, we allow the distribution process to proceed and at the selected time after the injection we harvest the specimen and drop it in a slurry of dry ice in acetone.

Figure 15 [10] shows a cryostatic micro-CT image of a pig's coronary artery that was harvested and snap frozen at the end of an intracoronary dye injection. Notice the lumen, vasa vasorum and the halo of non opacified tissue – where the dye had not yet diffused from the main lumen and there are no vasa vasorum to deliver it as they do in the outer layer of the arterial wall.

Figure 16 [11] shows how we analyze the such micro-CT images to extract the diffusion/transport information.

Figure 17 [11] - This shows that the increase in opacity due to diffusion of the contrast agent across the endothelial lining of the coronary artery is essentially uniform across the wall at some time after the intra arterial contrast injection is completed. Interestingly the amount of contrast is increased with hypercholesterolemia- suggesting that the endothelium becomes more leaky.

Figure 18 [11] - If we biopsy the tissue at sequential time points we can build up a time curve as shown here. It shows the initial diffusion of the dye in the outer wall from the vasa vasorum and also next to the main lumen. Then as time progresses we get a movement of the dye from the main lumen towards the outer wall as it is removed via the venous vasa vasorum.

Figure 19 [12] - CT images are of limited contrast and this can be helped by injecting contrast agents. It can also be extended by doing histology after the specimen has been scanned. Well registered images of histology and CT can tell us whether some vessels have not been filled and they can also tell us something about biologically active molecules in relationship to the vasculature – as shown by antibodies etc.

Figure 20 [13] - We can also use the histology to tell us something about the type of tissue so that we can assign material properties to those various tissues for subsequent finite element analysis of the mechanical behavior of the arterial wall during a distending pressure. In this case,

there was a local accumulation of fatty material, which introduces regions of localized stress and results in localized protrusion into the lumen.

Another approach is to selectively block micro-arteries with microspheres of known diameter,

Figure 21 [14] shows regions of absent perfusion down stream to embolizing microspheres – this occurs if the contrast injection is made after the embolization. If the embolization occurs immediately after the injection is finished, then the dye is trapped because it cannot washout from those lumens without flow. The volume of these territories can be measured from the 3D images from which the frequency distribution of their size can be derived.

We have found that the deleterious effect of micro-embolization is much greater than the death of muscle volume alone would suggest. The volume clearly increases linearly with embolization dose as shown by the bars, but the surface area differs little for the different size microspheres, suggesting it is the surface area and not the volume of muscle not perfused that is a problem little. A new idea about the goal of micro-CT analysis is to characterize the Basic Functional Units, the BFUs, rather than try to characterize the cells. A BFU is the smallest assembly of diverse cells that functions like the organ. Examples include the hepatic lobule and renal glomerulus and its nephron. They are 100-200  $\mu\text{m}$  in diameter. Clearly, it would be impractical to image all the cells in an organ, but it is practical to image all  $10^6$  BFUs that make up an organ.

Figure 22 [15] shows that we can image the vessels and all the BFU glomeruli in a rat kidney, and Figure 23 [17] detailed images of the glomerulus and tubule that make up the kidney's BFU.

Figure 24 [16] - There are a number of imaging problems that affect analysis of vascular trees. Thus blurring due to the imaging system, e.g. focal spot diameter, and partial volume effects of the voxelization process in the image reconstruction as well as noise. These effects are increasingly serious as the vessels becomes smaller, so a systematic error could be made.

The blurring can be overcome in large measure by Point Spread Function deconvolution – but at the price of noise.

Figure 25 [17,18] - Due to technological limitations and cost considerations the imaging system may not image the entire cross section of the structure of interest. Several algorithms have been developed to at least partially overcome these problems. One is profile extension, one is local reconstruction, and one is super resolution. Contrast agent properties are of particular interest. New contrast agent that do not leave the vascular lumen by diffusion and are not excreted by the kidney are becoming available. This means that gated imaging of the cardiovascular system of living animals is greatly facilitated.

Another problem is the huge data load that must be analyzed. Thus, automated analysis of the arterial tree is mandatory – yet no one has completely solved this problem as far as I know.

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